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(54) A process for the preparation of highly monodispersed polymeric hydrophilic nanoparticles

(57) The invention relates to a process for the preparation of highly monodispersed polymeric hydrophilic nanoparticles. The particles have a size of upto 100 nm. The nanoparticles of the present invention may have drug substances encapsulated therein. The process comprises in subjecting a mixture of an aqueous solution of a monomer or preformed polymer to reverse micelles a cross-linking agent, initiator. The mixture may additionally contain a drug or target substance. The polymerised reaction product is dried for removal of solvent to obtain dried nanoparticles and surfactant employed in the process of preparing reverse micelles. The dry mass is dispersed in aqueous buffer and the surfactant and other toxic material is removed therefrom.

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Description

This invention relates to a process for the preparation of highly monodispersed polymeric hydrophilic nanoparticles with or without target molecules encapsulated therein and having sizes of upto 100 nm and a high monodispersity.

PRIOR ART

Following an administration of a drug in a living system, the active substance is distributed throughout the body as a function of its physiochemical properties and molecular structure. The final amount of drug reaching its target site may only be a small fraction of the administered dose. Accumulation of drug at the non-targeted site may lead to adverse effect and undesirable side reactions. Therefore, targeting of drug to specific body sites is necessary.

One way of modifying the biodistribution of drugs in the body is to entrap them in ultrafine drug carriers. Among these carriers, liposomes, nanoparticles and pharmacosomes have been extensively studied. The use of liposomes as drug targeting agents is found to be limited due mainly to the problems of low entrapment efficiency, drug instability, rapid drug leakage and poor storage stability. With the aim of overcoming these problems, the production of polymeric nanoparticles has been investigated since the last two decades. Nanoparticles are defined as solid colloidal particles ranging in size from about 10nm to 1000nm.

A large number of studies have been reported on recent advances in drug targeting possibilities and sustained release action with nanoparticles encapsulating drugs. In vivo studies have also been reported with special attention to the reticuloendothelial system (RES). Some in vivo studies concerning nanoparticles administration by oral and ocular routes have also been reported in the literature with respect to the possible improvements of bioavailability. These polymeric nanoparticles should be non-antigenic, biocompatible and biodegradable.

The important characteristics of the particles used for targeting at specific body sites were found to be influenced mainly by two factors: (i) the size of the nanoparticles and (ii) the surface characteristics of the nanoparticles.

Particles smaller than 7 μm and specially nanoparticles are not filtered in the lung and their biodistribution is dependent on their interaction with reticuloendothelial system (RES). Biodegradable nanoparticles are mainly taken up by the Kupffer cells in the liver while small amount of these particles go to macrophages in spleen and bone marrow. Bone marrow uptake and targeting at other sites can be modified drastically by reducing the particle size. Nan particles of 200nm diameter and above have biodistribution dependent on their interaction with RES. The distribution, however, can be reversed if the particle size is made much smaller (ie. below 100nm) and particle surfaces are made hydrophilic. As an example, it has been found that if these particles are separated into three size ranges - 60nm, 150nm and 250nm and their surfaces are rendered hydrophilic by adsorbing poloxamer type of surfactants, then the small sized particles with maximum surface hydrophilicity are mostly taken up by cells other than Kupffer cells and as shown in Fig.1 of the accompanying drawings. Specifically, Fig.1 shows size dependent uptake of nanoparticles, uncoated and coated with poloxamer surfactant by bone marrow. These small particles in the blood serum do not adsorb serum protein through opsonisation and as a result, their circulation time in blood is considerably increased. Hydrophobic particles are removed from the circulation very rapidly due to opsonisation. Nanometer sized particles with hydrophilic surface remain in blood for longer period of time so that targeting at specific sites may be facilitated.

At present, nanoparticles for drug encapsulation are prepared by methods involving either polymerisation of dispersed monomers or a dispersion of preformed polymers in emulsion in presence of desired drug. The methods known in the art for the preparation of nanoparticles are (i) dispersion polymerisation method, (ii) emulsion polymerisation method, (iii) dispersion of synthetic polymer nanospheres in emulsion, and (iv) interfacial polymerisation technique. In all these methods, emulsions of oil-in-water are used and the polymer is formed or dissolved in the oil phase.

As a result, the polymeric materials are always hydrophobic because they are to be soluble in oil and the particles formed are nanoparticles of larger size (ie. above 100nm) because the average size of the emulsion droplets is mostly 100nm and above diameter. Moreover, since the emulsion droplets are highly polydispersed, the nanoparticles formed have broad spectrum size range and these are also highly polydispersed. Thus, in such known processes (i) one cannot prepare nanoparticles of subcolloidal size and (ii) the emulsion medium demands that the polymeric materials should be hydrophobic.

OBJECTS OF THE INVENTION

An object of this invention is to propose a novel process for the preparation of highly monodispersed polymeric nanoparticles with or without targeted materials and having a size of upto 100nm with a high monodispersity.

A further object of this invention is to propose a process for the preparation of said polymeric nanoparticles capable of being modulated to required sizes.

Another object of this invention is to propose a process for the preparation of said highly monodispersed polymeric nanoparticles of subcolloidal size with or without targeted materials.

Still another object of this invention is to propose a process for the preparation of said hydrophilic polymeric nanoparticles.

A further object of this invention is to propose a process for the preparation of said highly monodispersed drug loaded polymeric nanoparticles dispersed in aqueous buffer and free of any toxic material.

A still further object of this invention is to propose a process for the preparation of highly monodispersed drug loaded polymeric nanoparticles of hydrophilic nature which obviates the disadvantages associated with those of the prior art.

Yet another object of this invention is to propose a process for the insertion and loading of target drug/target substance in nanoparticles to secure them from outer intervention in vivo or cell culture invitro till they are exposed at the target site within the cell.

DESCRIPTION OF INVENTION

According to this invention, there is provided a process for the preparation of highly monodispersed polymeric hydrophilic nanoparticles with or without targeted materials having a size of upto 100nm with a high monodispersity comprising in the steps of :

(i) dissolving a surfactant in oil to obtain reverse micelles;
(ii) adding an aqueous solution of a monomer or preformed polymer to said reverse micelles and a cross-linking agent, initiator and drug or a target substance, if required;
(iii) subjecting such a mixture to the step of polymerization;
(iv) drying the polymerized reaction product for removal of solvent to obtain dry nanoparticles and surfactant;
(v) dispersing the dry mass in aqueous buffer; and
(vi) separating the surfactant and other toxic materials therefrom.

In accordance with this invention, the aqueous core of a reverse micellar droplet is used as a nanoreactor for the preparation of nanoparticles. The sizes of the aqueous core of such droplets are in the range of 1nm-10nm. The size of the particles which are formed primarily inside these droplets are larger than the size of the aqueous core of the droplets. Moreover, since the polymerisation takes place in an aqueous medium, polymers with surface hydrophilic properties are obtained by this invention. Therefore, using reverse micellar method of the present invention, it is possible to prepare very small size nanoparticles with hydrophilic surface so that their opsonisation as well as uptake by RES is substantially minimized. High monodispersity of the particles is possible because reverse micellar droplets in which the polymeric reactions are carried out are highly monodispersed.

The aqueous phase is regulated in such manner so as to keep the entire mixture in an optically transparent micro emulsion phase. The range of the aqueous phase cannot be defined as this would depend on factors such as the monomer, surfactant or polarity of oil, and the only factor is that the system is in an optically transparent micro emulsion phase.

In accordance with the present invention, the nanoparticles have a size range of upto 100nm, preferably a size of upto 10nm to 100nm.

In accordance with this invention, the aqueous core of a reverse micellar droplet is effectively used as nanoreactor to prepare ultrafine nanoparticles and to encapsulate the drugs (normally water soluble chemicals of maximum size upto that of 100-200 k Dalton protein. By the process of the present invention, extremely small particles of size of greater uniformity and down to about 10nm diameter has been achieved.

The surfactant, sodium bis ethylhexylsulphosuccinate, or Aerosol OT (ie. AOT) is dissolved in n-hexane to prepare reverse micelles. To the AOT solution in hexane (usually 0.03M to 0.1M of AOT in hexane), aqueous solutions of monomer or preformed polymer, cross-linking agent, initiator and drug are added and the polymerisation is done in presence of nitrogen gas. Additional amount of water may be added in order to get nanoparticles of larger size. The maximum amount of drug that can be dissolved in reverse micelles varies from drug to drug and has to be determined by gradually increasing the amount of drug till the clear microemulsion is transformed into translucent solution. All the stock solutions are prepared in phosphate buffer and the contents swirled vigorously in order to ensure the transparency of the solution. The reaction mixture is purged with nitrogen gas. Polymerisation is done in nitrogen atmosphere. The solvent n-Hexane is then evaporated out at a temperature, for example, of 35°C using rotary evaporator under low pressure when transparent dry mass is obtained. The material is dispersed in water and to it CeC12 solution is added, drop by drop till all the calcium salt of diethylhexylsulphosuccinate (Ca(DEHSS)2 from AOT) is precipitated. The mixture is then subjected to centrifugation, for example, at 15,000 rpm for 10 mins.. The supernatant is decanted off which contains nanoparticles containing encapsulated drug. Some nanoparticles remain absorbed in the cake of the precipitate. For complete recovery of the nanoparticles from the precipitated calcium (DEHSS)2, the latter is dissolved in n-hexane and the nanoparticles extracted with water. The aqueous dispersion is immediately dialysed through, for example, 12,000 cut off dialysis membrane for about one hour and the liquid ly-ophilised to dry powder and stored at low temperature till further use.

Reference is now made to Fig.2 of the accompanying drawings which illustrates the flow diagram for the preparation of nanoparticles using microemulsion. Step A shows a reverse micellar droplet A1, prepared by dissolving the surfactant in oil. Thus, when a surfactant is

dissolved in oil, then the hydrophobic constituent tails A2 would remain in contact with oil and an inner core A3 would comprise of hydrophilic constituents. When water is added to a solution containing reverse micelle A1 of as the hydrophilic constituent is soluble in water, water is attracted to the hydrophilic domain or core A3. A monomer cross-linking agent, the required drug and initiator is added to the reverse micelle A1. As the aforesaid constituents are hydrophilic in nature, such constituents go to the core A3. Polymerization is carried out in nitrogen atmosphere to form a polymer B1 and encapsulated drug as shown in step B. In step B, an evaporation is effected under low pressure for removal of the solvent. Step C illustrates the dried mass to consist of nanoparticles C1, and surfactant C2. The dried mass is dissolved in phosphate buffer and then 30% CaC12 added thereto drop by drop in step D to precipitate the surfactant as calcium diethylhexylsulfosuccinate (DEHSS). Step D illustrates the nanoparticles C1 and calcium DEHSS. The solution of step D is centrifuged at step E to obtain clear nanoparticles dispersed in buffer and the precipitate of Ca(DEHSS)2.

The cake of Ca(DEHSS)2 may contain some absorbed nanoparticles which can be recovered by dissolving the cake in hexane and leaching the nanoparticles by buffer 2 to 3 times. The leaching solutions are collected along with solution E. Such a buffer solution containing nanoparticles may still contain certain unreacted or toxic materials which are removed by dialyzing the solution for two hours and then freeze dried.

Normally, 0.01 to 0.1M AOT in n-hexane is used. Vinylpyrrolidone (VP) or mixture of vinylpyrrolidone and polyethyleneglycolfumarate (PEGF) are used as monomers as they form water soluble hydrogels on polymerisation and are highly biocompatible. Another suitable but antigenic polymer which has been used is bovine serum albumin. Other suitable water soluble hydrogels and biocompatible materials can be used for nanoparticle formation. In case of hydrogels, the cross-linking is done with N,N methylene bis acrylamide (MBA) whereas albumin is crosslinked by glutaraldehyde. In case of polyvinylpyrrolidine (PVP) crosslinked with MBA, the amount of monomer used is, for example, about 50 w% of AOT, and the amount of cross-linking agent (MBA) used is 1.2% w/w of the polymer. Such a composition has maximum shelf life and retention of drug by nanoparticles of this composition is also maximum. Loading of drug should be between 1% to 10% w/w of the polymer according to the solubility of the drug in the micellar system but it can also be increased if the solubility of the drug in reverse micelles is high.

The following examples are given by way of illustration of the present invention and should not be construed to limit the scope of the present invention.

Example I : Preparation of an antigen loaded polyvinylpyrrolidone nanoparticles.

An antigen, from Aspergilus fumigatus, has been used as a drug for encapsulation. In a 40ml of 0.03M AOT solution in hexane, 140µl of freshly distilled pure vinylpyrrolidone 35µl of N,N methylene bis arylamide (0.49mg/ml), 20µl of 1% ferrous ammonium sulphate solution, 40µl of 11.2% aqueous solution of tetramethylethylenediamine (TMED), 10µl of 5% potassium persulphate as initiator and 180µl of antigen (antigen =16mg/ml) were added. The amount of excess buffer to be added in reverse micelles was governed by the desired size of the nanoparticles to be prepared. The volume of the excess buffer can be carried from zero to maximum amount upto which microemulsion formation is possible and no phase separation takes place. The solution was homogeneous and optically transparent. Polymerisation was done in presence of N2 gas at 30°C for 8 hours in a thermostatic bath with continuous stirring. The nanoparticles of polyvinylpyrrolidone containing encapsulated drug would be formed. The solvent was evaporated off in a rotary vacuum evaporator and the dry mass was resuspended in 5ml of water. Calculated amount of 30% CaC12 solution was added drop by drop to precipitate AOT as calcium salt bisethylhexysulphosuccinate. The centrifuged aqueous solution contains nanoparticles which was homogeneous and almost transparent. The cake of calcium DEHSS after centrifugation contains some amount of nanoparticles absorbed in it. It was dissolved in 10ml of n-hexane and the hexane solution was washed 2-3 times each time with 1ml water. The phase separated clear aqueous layer was drained out and was collected with the original filtrate. The total aqueous dispersion of nanoparticles was then dialysed (12,000 cut off membrane) for about 2 hours against water and the dialysed solution was lyophilised immediately to dry powder for subsequent use. The sample should be free from AOT, monomer, cross-linking agent and perdisulphate. Any trace amount of unreacted materials and surfactant could be detected through HPLC. Perdisulphate was detected chemically using starch iodide solution and the presence of AOT was tested as follows :

To a 1mg/ml solution of dry powder, a drop of methylene blue dye was added. The solution was then mixed with 1ml of n-hexane thoroughly and was kept for phase separation. The hexane layer was then tested spectrophotometrically at 580nm for the presence of the dye.

Example II : The nanoparticles from polyethyleneglycolfumarate were prepared as follows :

5g of polyethylene glycol 600, 0.9g of fumaric acid and 1.22 mg of hydroquinone were mixed together and heated at 190°C for 7-8 hours in a 100ml 3-necked flask equipped with a thermometer, refluxing condenser and a nitrogen inlet. The product was of greenish yellow vis-

cous liquid at room temperature.

In a 40ml of 0.06m AOT n-hexane the following components were added. 100µl of polyethyleneglycol fumarate (0.186g/ml), 10µl of freshly distilled vinylpyrrolidone, 10µl of N,N methylene bis acrylamide (0.049g/ml), 10µl of 0.5% ferrous ammonium sulphate, 20µl of 11.2% TMED and 10µl or 20µl, as the case may be of fluorescence isothiocyanate-dextran (FITC-dextran) of mol. wt. 16KD (160mg/ml). 0-200 µl of buffer depending on the size of the droplet were added.

In the above solution, N2 gas was passed for 30 mins. and then 10µl of 5% potassium perdisulphate was added as initiator with vigorous stirring. Thereafter, the nitrogen gas was passed through the solution for another six hours at 30°C.

The nanoparticles were recovered from the aqueous solution following the same method as described earlier in the case of polyvinylpyrrolidone particles.

Example III : Preparation of Bovine Serum Albumin-glytaraldehyde nanoparticles.

In a 40 ml of 0.06m AOT in n-hexane 200µl bovine serum albumin (100mg/ml) and 0-600µl water depending on the desired size of the micellar droplets were added. The mixture was thoroughly stirred at room temperature till a transparent microemulsion was formed. To the well stirred solution, 20µl 5% glutaraldehyde was added and the stirring was continued for another half an hour when the nanoparticles were formed. The aqueous solution of the nanoparticles were prepared from the AOT solution following the method as described in the case of polyvinylpyrrolidone above.

The nanoparticles were characterised as follows :

The entrapment efficiency of the FITC-dextran dye in polyvinylpyrrolidone nanoparticles was determined as follows : The aqueous extract including the repeated washings were collected and was made up to the volume of 10ml. 500 µl of the solution was filtered through 100KD membrane filter and 2 µd phosphate buffer was added. The absorbance of the solution was measured at 493nm. The absorbance of the same concentration of free FITC in phosphate buffer was measured. From the difference in absorbances, the entrapment efficiency was calculated and the values as shown in the table was found to be in the range of 39-44% irrespective of the size of the nanoparticles.

Size of the PVP particles (nm)	Entrapment Efficiency (%)
21	40
26	44
31	42

(continued)

Size of the PVP particles (nm)	Entrapment Efficiency (%)
34	40
52	39
96	40

The size of the nanoparticles was determined by laser light scattering measurements.

Dynamic laser light scattering measurements for determining the size of the nanoparticles were performed using Brookhaven 9000 instrument with BI200SM goniometer. Argon ion air cooled laser was operated at 488nm as a light source. The time dependence of the intensity autocorrelation function of the scattered intensity was derived by using 128 channel digital correlator. Intensity correlation data was processed by using the method of cumulants. The translational diffusion coefficient (σT) of the particles dispersed in aqueous buffer was obtained from a non-linear least square fit of the correlation curve using the decay equation. From the value of the translational diffusion coefficient, the average of hydrodynamic diameter Dh of the scattering particles was calculated by Stokes-Einstein relationship

$$D_h = kT/3\bar{A}\eta\sigma T$$

where k is Boltzmen constant, η is the viscosity of the solvent at an absolute temperature T.

The size of the drug loaded nanoparticles of polyvinylpyrrolidone, polyethyleneglycolfumarate and bovine serum albumin were determined and representative spectra for each type are shown in the Figure 3(a). Fig.3(a) (i to iii) show

(i) nanoparticles made of polyethylene glycol fumarate containing FITC-Dextran
(ii) nanoparticles made of polyvinylpyrrolidone containing FITC-Dextran,
(iii) nanoparticles made of bovine serum albumin crosslinked with glutaraldehyde.

Fig.3(b) shows the variation of particle size with the change of size of the microemulsion droplets. Interestingly, the size of the polyvinylpyrrolidone nanoparticles increases exponentially with the increase of droplet size whereas the same remain more or less constant in case of bovine serum albumin-glutaraldehyde particles.

In vitro release kinetic studies :

A known amount of lyophilised nanoparticles encapsulating FITC-dextran was suspeded in 10ml of phosphate buffer saline in 50ml polypropylene tubes. The tubes were placed in water bath maintained at 37°C. At predetermined intervals, a volume of 500µl taken from each tube was passed through a 100KD filter (Millipore

UFP2THK24) which retained the nanoparticles and the free dye came out in the filtrate. The dye concentration in the filtrate was determined spectrophotometrically.

The results are shown in Fig. 4, which illustrates the release of FITC-dextran dye from polyethylene glycol fumarate particles of different loading, and where curve X1 shows a 6.4% loading and curve X2 is 3.2% loading of dye.

In vivo antibody response in mice serum by injecting antigen encapsulated nanoparticles.

Mice were injected subcutaneously three times at an interval of 7 days with PVP nanoparticles containing 300μg of Aspergillius fumigatus antigen entrapped in the PVP nanoparticles and suspended in 100μl of normal saline. Each group contains five animals, three of which received the antigen entrapped in nanoparticles, one received free antigen (300μg) and the control received empty nanoparticles suspended in normal saline. Mice were bled at predetermined intervals and the amount of Aspergilus fumigatus specific antibody in the mice serum was assayed using indirect ELISA assay. The results are shown in Fig.5, which illustrates specific antibody response.

Specific antibody response of antigen of Apergilus fumigatus entrapped in polyvinylpyrrolidone nanoparticles at different amount of cross-lining agent with curves X3,X4,X5 and X6 having 0%, 0.3%, 0.6% and 1.2% of cross-linking agent respectively.

Claims

1. A process for the preparation of highly monodispersed polymeric hydrophilic nanoparticles with or without targeted materials having a size of upto 100 nm with a high monodispersity comprising in the steps of :

   (i) dissolving a surfactant in oil to obtain reverse micelles;
   (ii) adding an aqueous solution of a monomer or preformed polymer to said reverse micelles and a cross-linking agent, initiator and drug or a target substance, if required;
   (iii) subjecting such a mixture to the step of polymerization;
   (iv) drying the polymerized reaction product for removal of solvent to obtain dry nanoparticles and surfactant;
   (v) dispersing the dry mass in aqueous buffer; and
   (vi) separating the surfactant and other toxic materials therefrom.

2. A pr cess as claimed in claim 1 wherein said nanoparticles have a size of 10nm to 100nm.

3. A process as claimed in claim 1 wherein said monomers and/or preformed polymers ar biocompatible and non-antigenic materials such as vinylpyrrolidone or mixture of vinylpyrrolidon and polyethyleneglycolfumarate, or their polymers such as polyvinylpyrrolidone or copolymer of polyvinylpyrrolidone and polyethyleneglycolfumarate.

4. A process as claimed in claim 1 wherein said polymers are biocompatible but antigenic such as bovine serum albumin.

5. A process as claimed in claim 1 wherein said cross-linking agent is N,N methylene-bis acrylamide (MBA) or glutaraldehyde.

6. A process as claimed in claim 1 wherein the said initiators are water soluble perdisulphate salts like ammonium perdisulphate and the activator is tetramethyl ethylene diamine (TMED).

7. A process as claimed in claim 1 wherein 1% to 10% target substance by weight of the polymeric material is encapsulated into said nanoparticles.

8. A process as claimed in claim 1 wherein 0.01M to 0.1M of surfactants such as sodium bis ethyl hexyl sulphosuccinate are used for reverse micelles preparation.

9. A process as claimed in claim 1 wherein said hydrocarbons are alkanes such as n-hexane.

10. A process as claimed in claim 1 wherein the dried nanoparticles and surfactant after removing hydrocarbon solvent are dispersed in buffer solution and then treated with calcium chloride to quantitatively remove the surfactant from the adhering nanoparticles.

11. A process as claimed in claim 1 wherein the nanoparticles dispersed in aqueous buffer is dialysed to remove the unreacted materials from the buffer.

12. A process as claimed in claim 1 wherein the dispersed nanoparticles after dialysis is lyophilised and preserved.

13. A process for the preparation of highly monodispersed polymeric hydrophilic nanoparticles substantially as herein described.

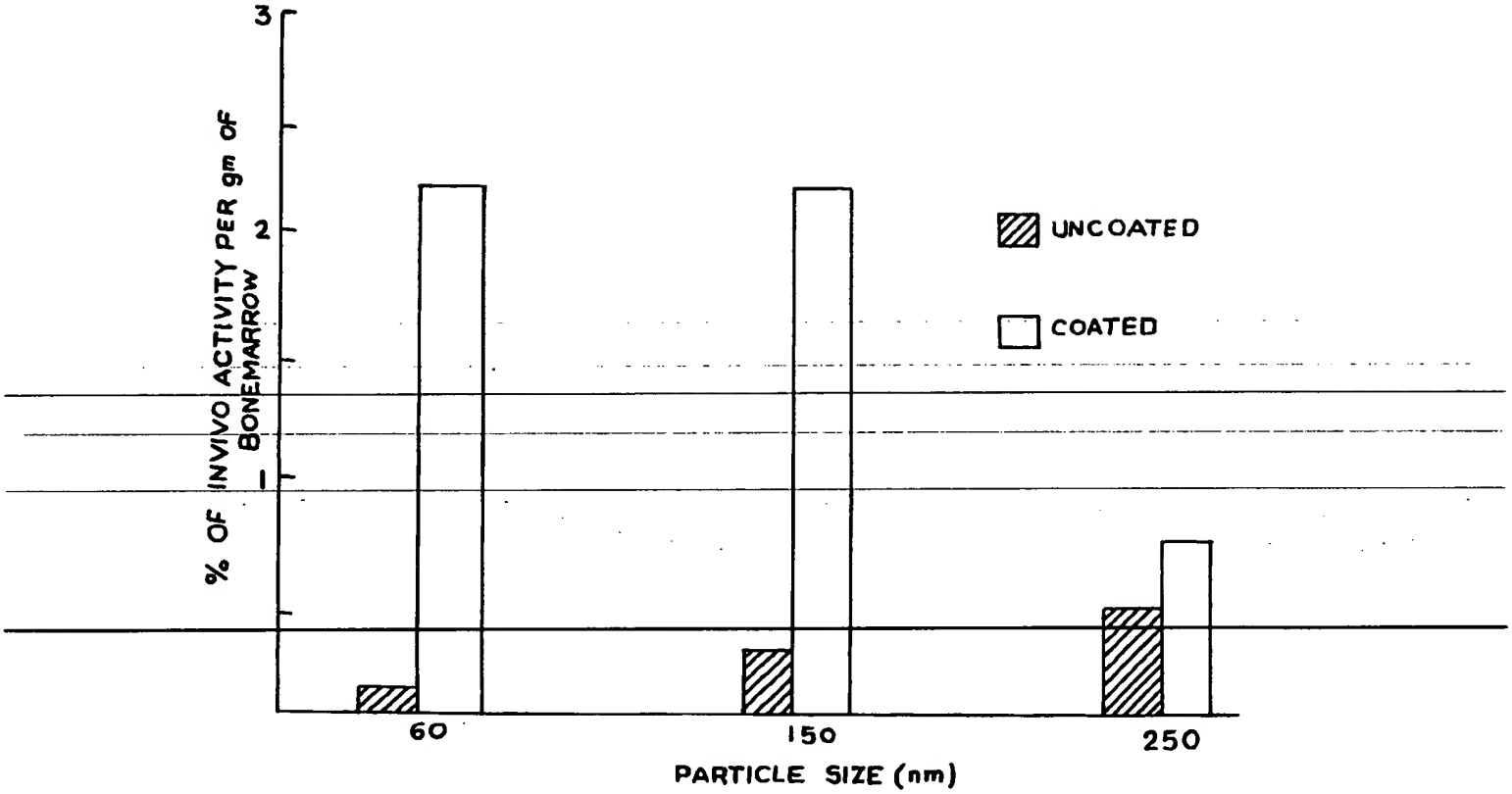
% OF INVIVO ACTIVITY PER gm OF BONEMARROW
3
2
1
UNCOATED
COATED
60
150
250
PARTICLE SIZE (nm)

Fig. 1

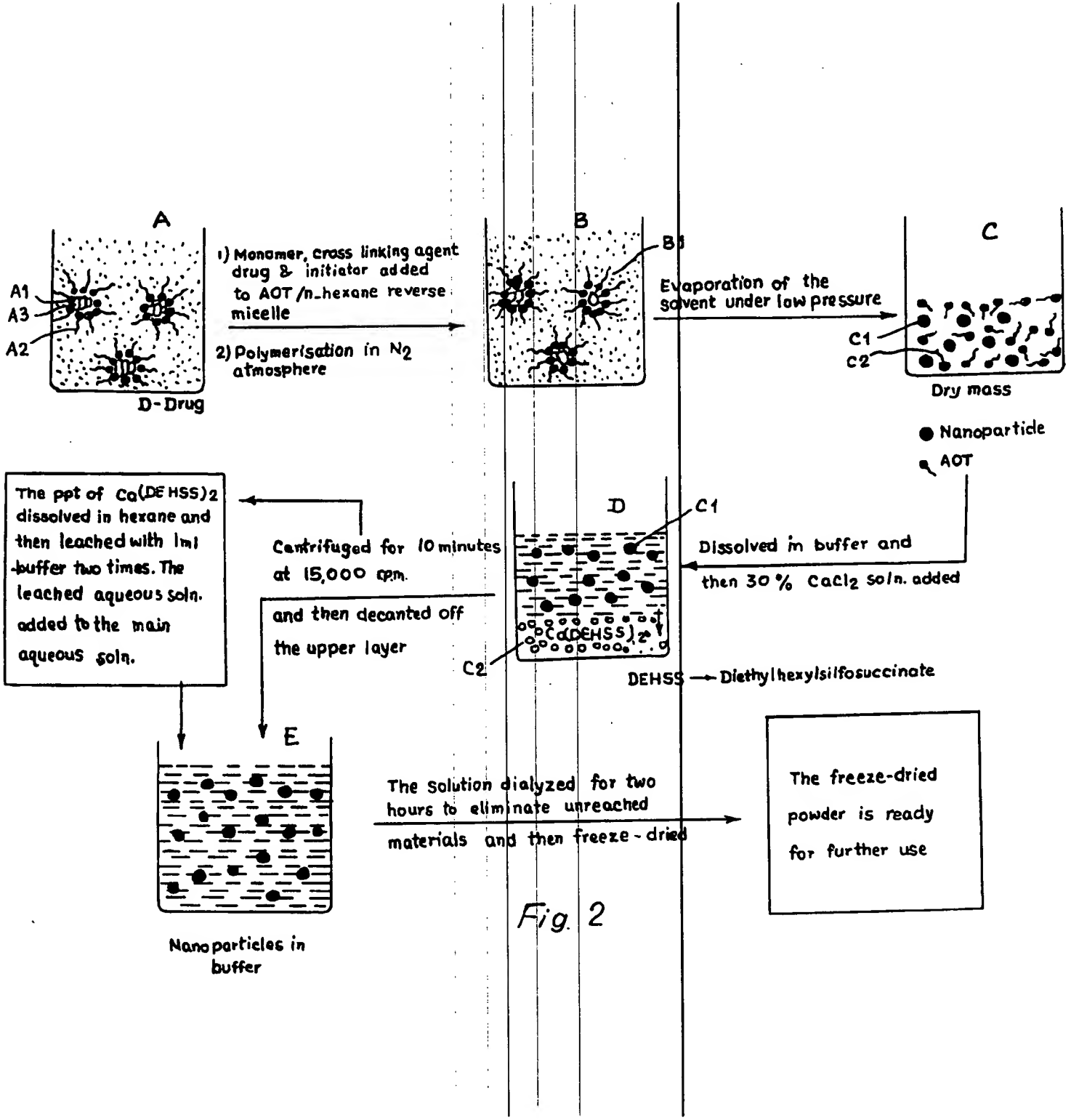
A
A1
A3
A2
D-Drug
1) Monomer, cross linking agent drug & initiator added to AOT /n-hexane reverse micelle
2) Polymerisation in N_2 atmosphere
B
B1
Evaporation of the solvent under low pressure
C
C1
C2
Dry mass
Nanoparticle
AOT
D
C1
Dissolved in buffer and then 30 % $CaCl_2$ soln. added
C2
$Ca(DEHSS)_2$
DEHSS → Diethylhexylsilfosuccinate
Centrifuged for 10 minutes at 15,000 rpm.
and then decanted off the upper layer
The ppt of $Ca(DEHSS)_2$ dissolved in hexane and then leached with 1ml buffer two times. The leached aqueous soln. added to the main aqueous soln.
E
Nanoparticles in buffer
The Solution dialyzed for two hours to eliminate unreached materials and then freeze-dried
The freeze-dried powder is ready for further use

Fig. 2

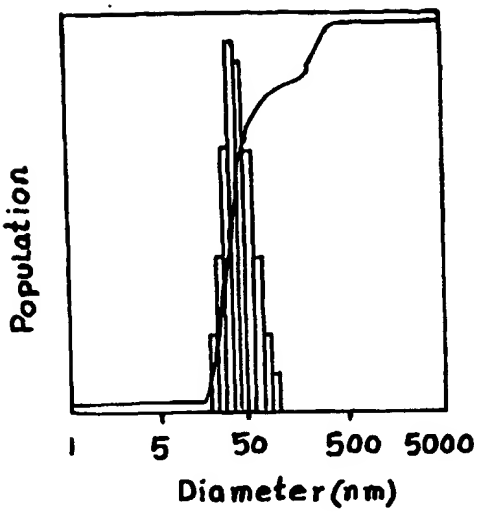

Fig. 3(a)(i)

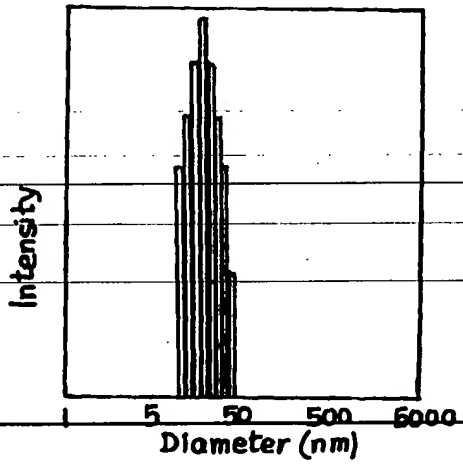

Fig. 3(a)(ii)

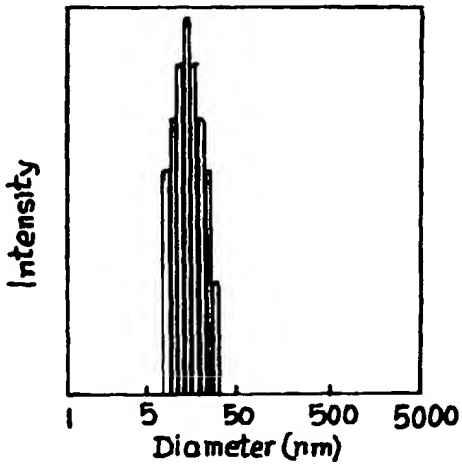

Fig. 3(a)(iii)

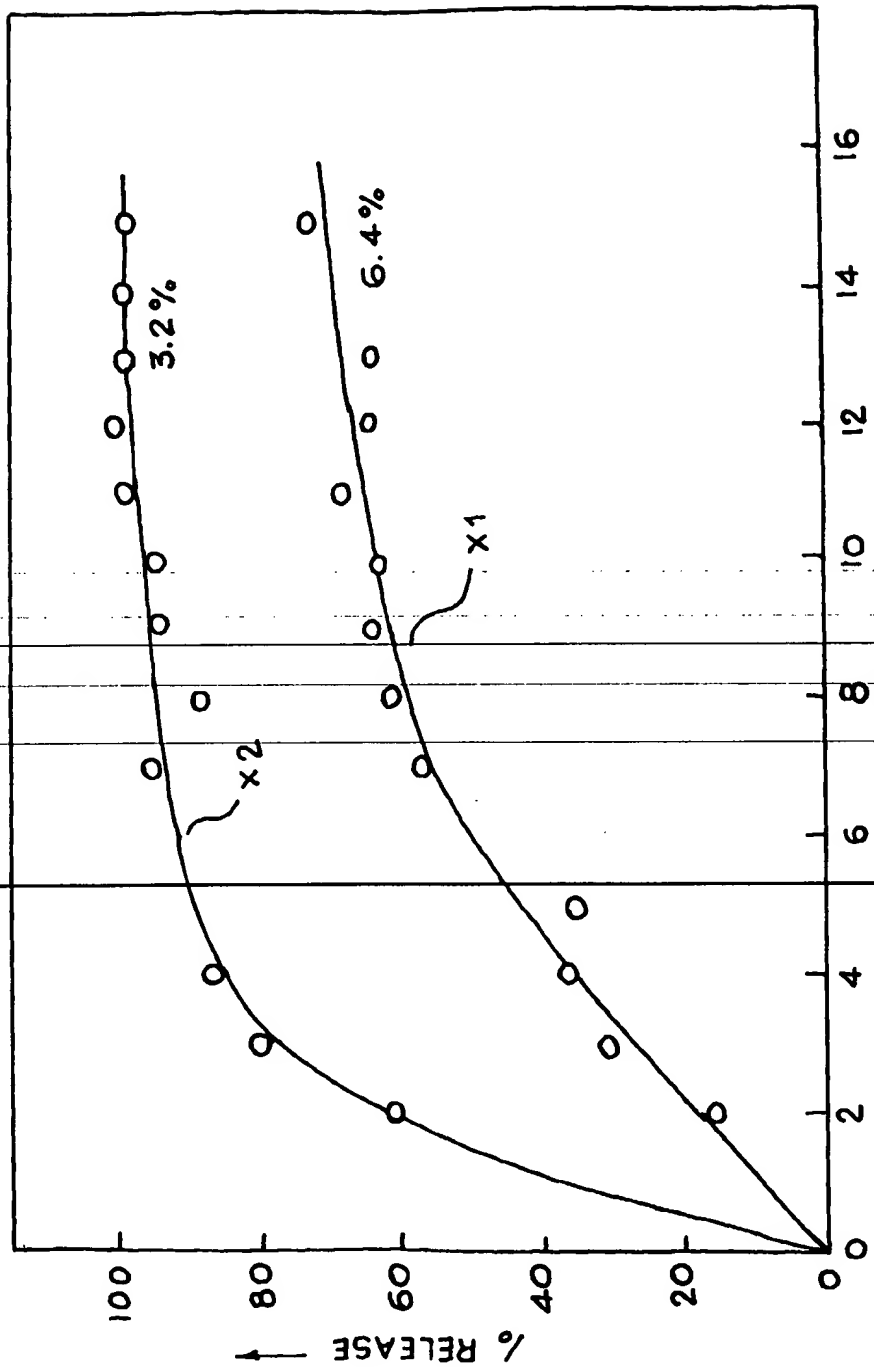

Fig. 4

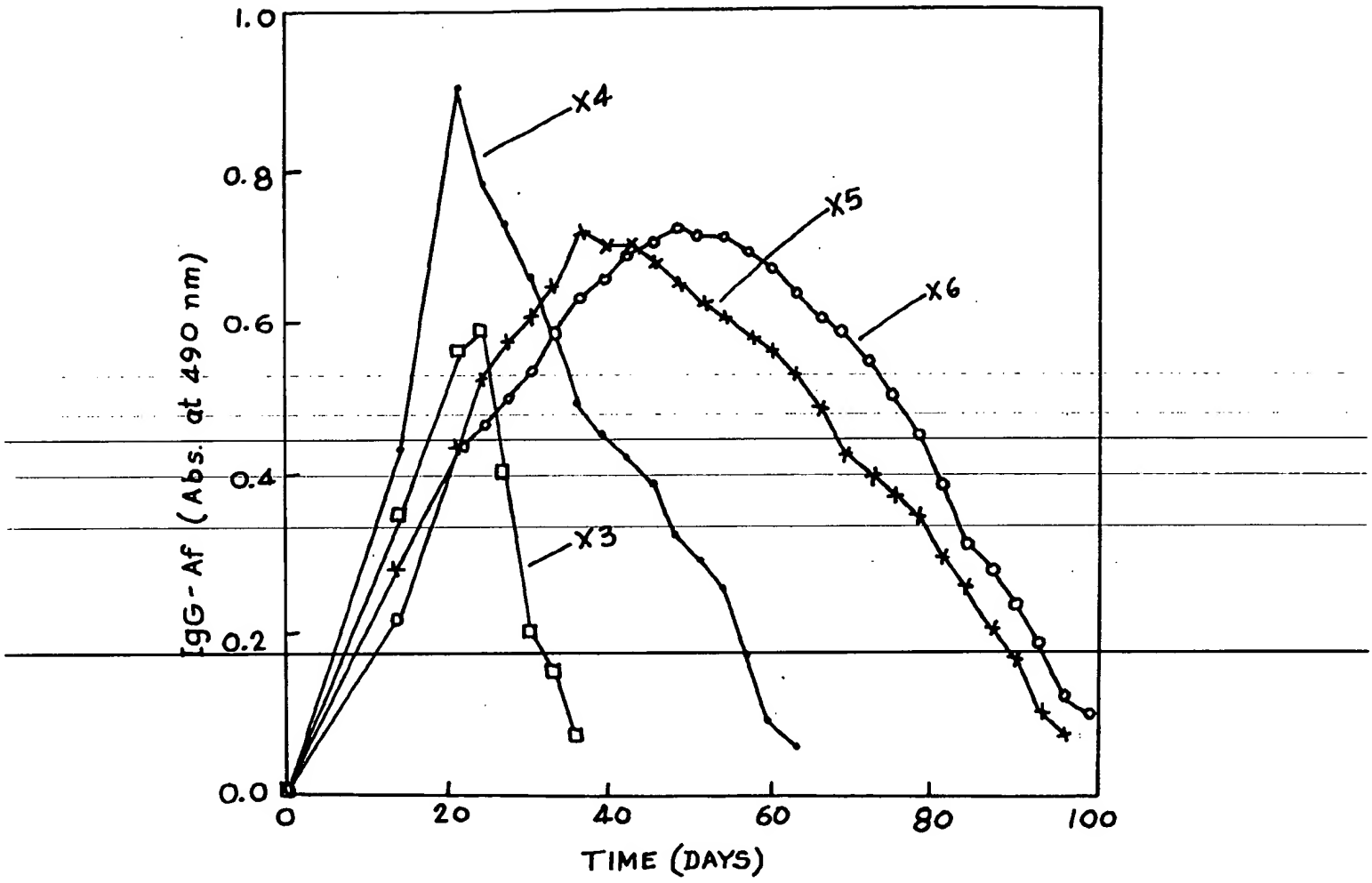
1.0
0.8
0.6
0.4
0.2
0.0
IgG-Af (Abs. at 490 nm)
0
20
40
60
80
100
TIME (DAYS)
X4
X5
X6
X3

Fig. 5

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 97 30 3127

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	FR 2 208 716 A (SPEISER PETER) * claims 1,5; example 1 * -----	1,5-13	C08F2/32 A61K9/51

TECHNICAL FIELDS SEARCHED (Int.Cl.6)
C08F A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	16 October 1997	Cauwenberg, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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